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# Combined effects of biochar and plant growth promoting bacteria *Pseudomonas putida* TSAU1 on plant growth, nutrient uptake of wheat, and soil enzyme activities

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**Abstract:** Many studies indicate the favorable effect of biochar on soil quality and plant fitness. A few studies on the biochar interactions with rhizosphere bacteria and their impact on plant development and nutrient acquisition have been reported. In this study, pot experiments in a glasshouse were performed to figure out the interactive effect of plant beneficial bacteria *Pseudomonas putida* TSAU6 and biochar amendment on the wheat (*Triticum aestivum* L.) growth, nutrient uptake, and soil biological activity. The results demonstrated that root and shoot dry biomass of wheat grown in soil mixed with biochar and combined with *P. putida* TSAU1 were enhanced significantly compared to the control plants. The nitrogen (N) and phosphorus (P) content in plant biomass were affected by biochar addition and biochar combined with a bacterial inoculant. It was also observed a change in soil enzyme activities, where higher fluorescein diacetate (FDA) and protease activities were observed after biochar application compared to control soil. The addition of biochar combined with bacterial inoculant showed a more profound effect on wheat growth and N, P acquisition. This positive effect is also associated with soil enzyme activities that are involved directly or indirectly in the N and P cycle. These findings confirm the potential of microbial inoculants induced by biochar to improve wheat production.

**Key words:** Maize biochar, nitrogen, phosphorus, dry plant biomass, nutrient uptake

## 1. Introduction

Biochar is a bioproduct made from biowaste, through pyrolysis under low or nonexistence of oxygen (Lehmann et al., 2011). Biochar showed several favorable impacts on soil and plant health, like enhanced soil organic matter content (Chan et al., 2007), soil enzyme activity (Ma et al., 2019a, 2019b), soil cation exchange and water holding capacity (Novak et al., 2009; Yu et al., 2013; Kul, 2022), microbial diversity (Egamberdieva et al., 2016; Egamberdieva et al., 2020a, 2020b), and plant nutrient acquisition (Cao et al., 2017). There were many reports on the positive effect of biochar application on plant growth, soil fertility, plant protection, and plant stress tolerance (Frenkel et al., 2017; Postma and Nijhuis, 2019). Such positive effect was explained by enhanced soil physical quality, soil water retention capacity, nutrient availability, and the microbial biodiversity involved in nutrient cycling (Kolton et al., 2011; Egamberdieva et al., 2017; Khan et al., 2021). Several reports showed higher microbial activity with plant beneficial traits in the plant root system grown in soil (Graber et al., 2010).

The plant beneficial effect of biochar and plant growth promoting rhizobacteria (PGPR) on plant growth and soil fertility has been well documented (Egamberdiyeva and Hoflich, 2002; Egamberdiyeva and Hoflich, 2003; Rondon et al., 2007; Mia et al., 2014; Abdelaal et al., 2022, Prakash et al., 2022). These application methods are considered environmentally friendly and allow lower inputs through reduced agrochemicals and pesticides that cause potential risks to the ecosystem (Wang et al., 2020). Several studies reported plant growth development by organic fertilizers, e.g., the plant growth of chickpea, and lupin (Egamberdieva et al., 2019; Egamberdieva et al., 2020b), maize (Islami et al., 2011), tomato (Graber et al., 2010), and wheat (Alburquerque et al., 2013) was increased by biochar application. PGPR promotes plant growth and nutrient uptake of various agricultural and medicinal plants (Alaylar, 2022), improves stress tolerance through traits

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such as phytohormones and hydrogen cyanide synthesis (Parray et al., 2016), cell wall degrading enzymes (Cho et al., 2015), antagonistic activity (Liu et al., 2019), ACC deaminase enzyme (Egamberdieva et al., 2011). However, a synergistic effect of biochar combined with PGPR in plant growth stimulation has been described in a few reports. Zafar-ul-Hye (2020) observed an improved plant growth of spinach by *Bacillus amyloliquefaciens* combined with biochar produced from compost. The biochar produced from wood combined with *Paenibacillus polymyxa* improved switchgrass growth and development (Shanta et al., 2016), and *Pseudomonas fluorescens* cucumber growth (Nadeem et al., 2017).

In this study, we aim to evaluate the combined impact of PGPR (*Pseudomonas putida*) on plant growth of wheat, nitrogen (N), phosphorus (P), and potassium (K) uptake and rhizosphere soil enzyme activities included in carbon (C), N, P cycle. The outcome of the research work will facilitate the development of strategies to enhance biochar effectiveness for wheat.

## 2. Materials and methods

### 2.1. Soil, plant, bacteria, and biochar

The soil samples were provided from the Experimental Station in Müncheberg, Germany. The soil contains 7% clay, 19% silt, and 74% sand. The chemical properties of the soil are containing: (C), nitrogen (N), phosphorus (P), potassium (K), and magnesium (Mg). The rate of these chemicals was 0.6%, 0.07 %, 0.03%, 1.25%, and 0.18% respectively and the pH was 6.2%. The maize was used to produce biochar (600 °C for 30 min) and the biochar contains 75.2% C, 1.6% N, 5.26% P, 31.2 K, and pH 8.9 (Reibe et al., 2015).

The plant growth stimulating bacteria *Pseudomonas putida* TSAU1 were taken from the Culture Collection of Microorganisms, Faculty of Biology, National University of Uzbekistan. In the previous experiments, the strain showed root and shoot growth stimulation of wheat grown in pots (Egamberdieva and Kucharova, 2009). The wheat seeds (*Triticum aestivum* L.) were exploited for pot experiments.

### 2.2. Plant growth experiment

The soil was mixed with 2% biochar. Wheat seeds were sterilized with 10% v/v NaOCl for two min, followed by 70% ethanol, and placed for germination in a dark room at 25 °C for two days. The bacterial strain *Pseudomoans putida* TSAU1 was grown in Tryptic Soy Broth (Difco Lab USA) for 2 days at 28 °C. Germinated sterile seeds were inoculated with bacteria and sown in pots added with 1000 g soil mixed with 2% biochar. The procedures were as follows: a) uninoculated plants grown in soil absence of biochar, b) uninoculated plants grown in soil presence of biochar, c) plants inoculated with *P. putida* TSAU1 and grown in soil without biochar; d) plants inoculated with *P. putida* TSAU1 and grown in soil amended with biochar. The four pots were used as replicates and placed in greenhouse with a temperature of 24 °C/16 °C (day/night). After 30 days, the dry biomass of root and shoot and Nitrogen (N) and phosphorus (P) concentrations were determined. N and P in dry biomass were analyzed using coupled plasma optical emission spectrometer (ICP-OES; iCAP 6300 Duo).

### 2.3. Soil enzyme measurements

The method of Green et al. (2006) was employed to investigate fluorescein diacetate (FDA) hydrolysis of soil. The acid and alkaline phosphomonoesterase activities in soil were determined following the method of Tabatabai and Bremner (1969). A p-nitrophenol (p-NP) was figured out from a p-NP calibration curve using a Lambda 2 UV-VIS spectrophotometer (400 nm, Perkin Elmer) (Acosta-Martínez, 2011). Ladd and Butler (1972) were used to determine soil protease activity.

### 2.4. Statistical analysis

As a variance analysis package, Microsoft Excel 2007 was exploited to analyze of statistical significance of data. A least significant difference (LSD) test ($p = 0.05$ was carried out for the determination of mean comparisons.

## 3. Results

### 3.1. Plant shoot and root growth

The effect of biochar combined with *P. putida* TSAU1 on the root and shoot dry weight of wheat were studied. It was clearly shown that the dry weight of root and shoot of wheat grown in soil amended with biochar, and biochar combined with *P. putida* TSAU1 were increased significantly confronted to the control plants. Root dry weights were 12% and 24% and shoot growth 24% and 36% higher in plants grown in soil amended with biochar and biochar combined with *P. putida* TSAU1 compared to control plants, respectively (Figure 1). No statistical differences in shoot and root growth between inoculated and uninoculated plants under both soil conditions were found.

### 3.2. Nutrient concentrations

The N, C, and P contents in plant dry biomass were influenced by the application of biochar alone and combined with *P. putida* TSAU1. The mineral concentrations were higher compared to plants grown in soil without biochar. A significant difference in N uptake over the controls was observed after biochar application, being 18% and 27% higher in plants grown in soil with biochar alone and combined with *P. putida* TSAU1, respectively (Figure 2A). The plants inoculated with *P. putida* TSAU1 showed a slight increase in N uptake (6%) by plants compared to the control plants, but the effect was not considerable (Figure 2A). The P uptake was significantly increased by 21% after biochar application compared to plants grown in soil without biochar (Figure 2B). An improvement of P uptake (10%) by plants inoculated with TSAU1 bacteria and grown in soil without biochar was observed, however, the effect was not significant. The combined treatment of bacterial inoculation with biochar showed a more beneficial effect on wheat P uptake. The P uptake of wheat inoculated with TSAU1 and grown in soil amended with biochar was significantly increased by 26% (Figure 2B).

### 3.3. Soil enzymes

Soil FDA hydrolase activity in soil was higher compared to soil without biochar. The FDA activity was increased by 33% and 42% in soil amended with biochar, and in soil amended with biochar inoculated with TSAU1, respectively. The soil derived from root system plants inoculated with bacteria showed a slight rise in FDA activity (Figure 3A). Biochar also increased soil protease activity (Figure 3B), i.e. an increase of 26% and 35% were sighted after biochar addition and in soil inoculated with TSAU1 compared to the control. The inoculated plants grown in soil without biochar demonstrated lower protease activity.

The biochar alterations influenced the acid and alkaline phosphomonoesterase activities under wheat soil (Figure 3C). There was a slight increase of alkaline phosphomonoesterase activity in soil collected from the root system of wheat inoculated with TSAU1, and no effect was observed in other treatments (Figure 3C). In contrast,

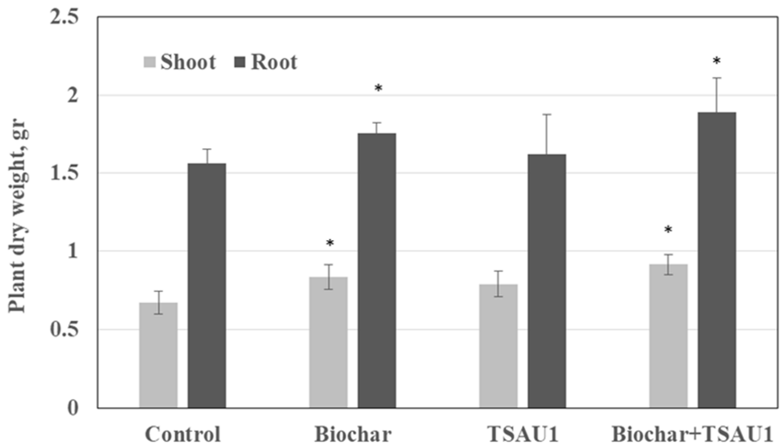


**Figure 1**. The shoot and root growth of wheat grown in soil amended with biochar and biochar combined with *Pseudomonas putida* TSAU1. The plants were uninoculated (control) or inoculated with TSAU1. Asterisks indicate the statistical significance of difference using Student's t test at $p < 0.05$.

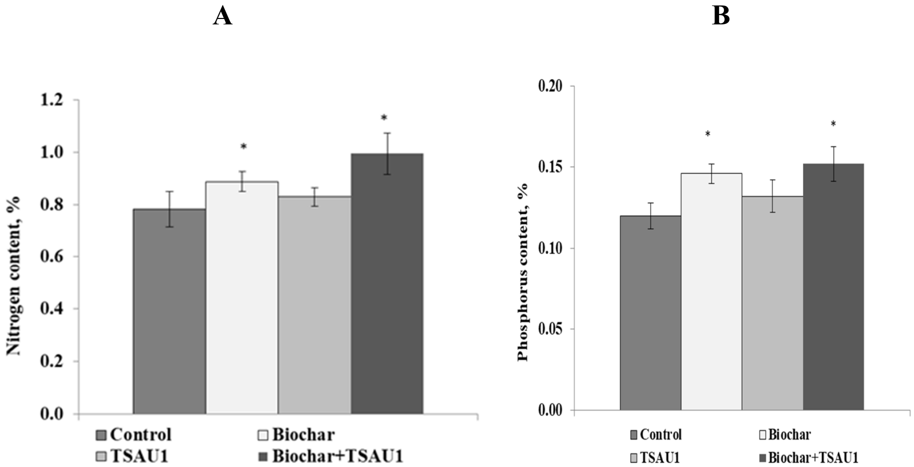


**Figure 2 (A–B).** Nitrogen (N) and phosphorus (P) concentration in wheat tissue grown in soil amended with biochar and biochar combined with Pseudomonas putida TSAU1. The plants were uninoculated (control) or inoculated with TSAU1. Asterisks indicate statistical significance of difference using Student's t test at $p < 0.05$.

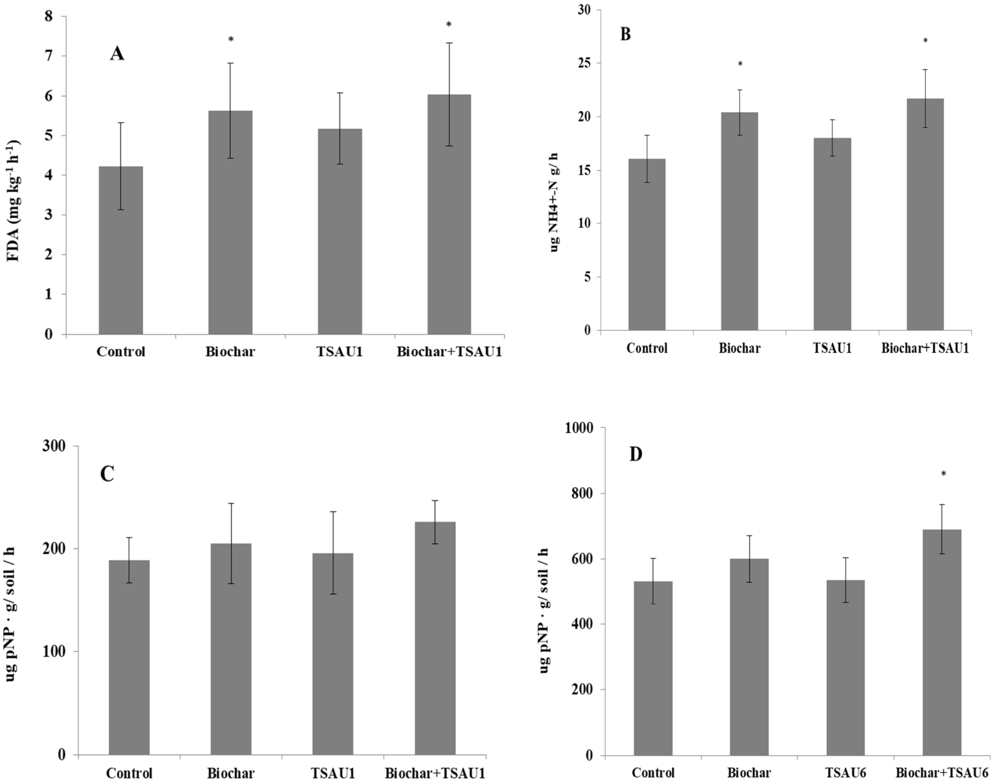


**Figure 3.** The effect of biochar and biochar combined with *P. putida* TSAU6 on soil FDA activity (A), soil protease activity (B), alkaline phosphomonoesterase (C), and acid phosphomonoesterase activity (D).

acid phosphomonoesterase activity was increased in soil amended with biochar by 12%, but no significant effect was observed (Figure 3D). The soil amended with biochar combined with plant inoculation with TSAU1 showed a significant increase of up to 29%.

## 4. Discussion

The current study reported that biochar has an important effect on plant microbe interactions, soil enzyme activities, as well as on nitrogen and phosphorus uptake of plants. The root and shoot biomass of wheat uninoculated and inoculated with *P. putida* TSAU1 were higher under biochar applied soil compared to plants grown in control. The improvement of plant microbe interactions under biochar application was reported in several studies (Wang et al., 2018; Egamberdieva et al., 2019; Egamberdieva et al., 2020a). Several studies explained this positive effect as facilitation of favorable conditions for bacterial proliferation, protection from abiotic stresses, and availability of nutrients by biochar (Pietikainen et al., 2000; Iijima et al., 2015). Besides, biochar provides benefits for root-associated microbial diversity that produces various plant growth stimulating metabolites (Quilliam et al., 2013; Egamberdieva et al., 2016; Shurigin et al., 2021). This finding indicates that biochar improves the potential effect of plant beneficial bacteria on plant growth, through possible stimulation of root system. The root associated bacteria produce various phytohormones, siderophores, hydrogen cyanide, ACC deaminase, and are able to solubilize phosphate (Cho et al., 2015; Egamberdieva et al., 2020b). Biochar addition into soil increases nutrient availability for microbial proliferation and thus increases their activities (Prendergast-Miller et al., 2014).

The biochar usage also enhanced N and P contents in plant tissues, whereas additional benefits were observed when biochar combined with a bacterial inoculant. Abdelaal et al. (2022) found that biochar applications promoted soil properties, soil pH and organic matters, and product yield of barley under drought conditions. The increased plant growth and P ratio in the plant tissue of wheat were also reported by Bista et al. (2019). Amini et al. (2016) noted the favorable effect of additional nutrients on the soil available for plant uptake, and development (Qayyum et al., 2012).

It has been also observed a change in soil-enzyme activities by biochar and bacterial treatment. In soil amended with biochar, higher FDA activity was observed compared to soil without biochar application. Similar data were given by Ma et al. (2019a), where biochar produced from black cherry wood enhanced FDA activity in soil under soybean. In our experiment more increase in enzyme activity was observed in soil collected from the root system of inoculated wheat grown in soil amended with biochar. Moreover, the soil sampled from the root system of wheat inoculated with bacteria showed higher FDA activity. A similar situation was recorded by Fall et al. (2016) where higher soil FDA hydrolytic activity was found in soil inoculated with microbes compared to uninoculated ones. Furthermore, biochar addition into soil increased soil protease activity, such increase could also be related to improved microbial community related to C, N, and P cycling activities (Wu et al., 2016; Shi et al., 2019). Wang et al. (2015) determined enhanced enzyme activities included in C and N cycles in soil amended with maize biochar.

The biochar combined with bacterial inoculants increased soil acid phosphomonoesterase activity, however, no changes were found in other treatments. It is likely that plant associated microbes included in organic P mineralization facilitate plant available P and increase its uptake by plants (Blackwell et al., 2010; Masto et al., 2013). There were no alters in soil alkaline phosphomonoesterase, except a slight increase in soil amended with biochar combined with bacterial inoculation.

## 5. Conclusion

Generally, biochar addition in soil combined with bacterial inoculation showed a more profound effect on wheat growth and nitrogen and phosphorus acquisition. This positive effect also correlated with soil enzyme activities contained in N and P cycle and also demonstrated the synergistic effect of biochar amendments on plant growth, plant nutrient uptake, and soil enzyme activities. These findings supply novel perspectives on the potential of microbial inoculants induced by biochar for the improvement of wheat production.

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## Conflicts of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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